

1368-Pos Board B319**Regulation of I_{Ca} during Simulated Acute Ischemia in Developing Cardiomyocytes Exposed to Hypoxia and Low pH**

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Ischemic heart disease is a common pro-arrhythmic condition characterized by hypoxia, acidosis, hyperkalemia and impaired Ca^{2+} handling. Here we investigated the ischemia responses to acute hypoxia and acidification in developing cardiomyocytes derived from neonatal rat hearts (rN-CM) or human induced pluripotent stem cells. L-type Ca^{2+} current (I_{Ca}) was measured within 4-7 days in the whole-cell perforated-patch (amphotericin B) configuration. Results: (1) Both cell types responded to acute hypoxia (5-100 s, $pO_2 < 5$ mmHg) with a maximum 40% suppression of I_{Ca} . (2) Higher levels of phosphorylation (100 nM isoproterenol) in both cell types caused a lower I_{Ca} blockade rate. (3) The hypoxic suppression in rN-CM showed variable kinetics: The hypoxic suppression of I_{Ca} stabilized at ~20% within ~10s in cells where inactivation of I_{Ca} occurred within 10 ms, while cells with slower inactivation of I_{Ca} ($\tau > 40$ ms) showed continued suppression up to 40%, that developed over tens of seconds and appeared to involve SR or mitochondria. (4) In both cell types the suppressive effect of acidosis (pH 7.4 to 6.7) on the quickly inactivating I_{Ca} was larger (~40%) and faster than the 25% observed for the slowly inactivating component of I_{Ca} . (5) The effects of hypoxia and acidosis were additive in cells with quickly inactivating I_{Ca} , but not in cells where I_{Ca} inactivated slowly. (6) Acidosis always accelerated the activation of the I_{Ca} by ~25%.

The results suggest that different mechanisms with distinct kinetics mediate the oxygen- and pH-sensing of the L-type Ca^{2+} channel, and consequently influx Ca^{2+} , handling of cytosolic Ca^{2+} by SR and mitochondria and the conditioning of the cells to acute hypoxic and acidosis stresses.

1369-Pos Board B320**Non-Linear Reduction of Ion Currents in Cultured Cardiac Myocytes: Correlation with a Loss of T-Tubules?**

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Background: Downregulation of K^+ currents and loss of T-tubule density is well documented during heart failure. A similar reduction of both has been observed in cultured ventricular myocytes. We examined whether the loss of K^+ and Ca^{2+} currents in cultured cardiac myocytes correlates with a loss of T-tubules.

Methods: Atrial and ventricular myocytes were isolated and cultured up to 48 hours. Current and voltage clamp recordings were made using patch electrodes. Confocal scans of myocytes stained with di-8-ANEPPS were made to visualize myocyte ultrastructure.

Results: Action potential (AP) recordings from control ventricular myocytes displayed a large phase 1 and spike-and-dome morphology. After 48 hours culture, the AP duration was greatly prolonged, and loss of the spike-and-dome morphology was noted. Voltage clamp analysis of I_{to} , I_{Kr} , and I_{Ca} revealed that the magnitude of all currents was significantly reduced after 48 hours in culture. However, I_{Kr} was reduced the largest after 48 hours (61% for I_{Kr} , 34% for I_{to} and 19% for I_{Ca}). I_{K1} was not significantly altered. Recovery from inactivation of I_{to} was greatly slowed in cultured ventricular cells. Confocal scans of T-tubule structure in ventricular cells showed a loss of T-tubules with prolonged days in culture. The loss of ion currents in ventricular cells may be related to a loss of T-tubules. We next measured I_{to} in atrial cells (which lack T-tubules) and found a downregulation of I_{to} when cultured or isolated from failing hearts.

Conclusion: In cultured ventricular myocytes, there was a non-uniform loss of ion currents similar to what is observed in heart failure. Moreover, the loss of ion currents in ventricular cells does not appear to correlate with a loss of T-tubules since atrial cells (which lack T-tubules) exhibited a similar loss.

1370-Pos Board B321**T-Tubules in Myocytes of Intact Dog Left and Right Atria**

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The cardiac ventricular T-tubule (TT) system has a major role in normal and abnormal E-C coupling. TT allow for balanced calcium cycling and synchronous contraction throughout the myocyte. However, there is some evidence that T-tubules are absent from atrial myocytes of large animals. The goal of this project was to analyze TT organization in normal intact dog atria. Whole left (LA) and right (RA) atria from dog hearts were perfused and maintained in a chamber on the stage of a confocal microscope. After loading with di-4-ANEPPS, we imaged endocardial myocytes from different atrial regions which were then analyzed using computerized analysis software. Many but not all myocytes had some TT although they tended to be arranged in clusters along one or both sides of the cell at very low density. In cells with TT, there was no difference in TT density between any regions of either atria. However, we did find RA had twice as many myocytes without TT than LA (25.1% of cells compared to 12.5%). These features of TT density and organization in dog atrium are dramatically different from ventricle (rat) where every cell has a highly dense and organized TT system. We also found that TT spacing was greater in LA than RA. Finally RA myocytes are wider than in LA so there is no correlation between cell width and TT density. We conclude that there are many cells in both dog atria with TT although many cells do not (more in RA than LA) and there are regional differences in TT spacing but not density in TT-containing cells within the atria. Thus, even though there is an organized TT system in dog atria, it is far less organized and dense compared to ventricle.

1371-Pos Board B322**Cdo Deficient Mice Display Cardiomyopathy with Alterations in Connexins**

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Shh signaling is required for various cardiac developmental processes including the looping of heart tube and specification of cardiomyogenesis. Consistently, Shh mutant mice display the abnormalities in heart tube formation and specification of the myocardial and outflow tract. A multifunctional receptor Cdo functions as a Shh coreceptor to fully activate Shh signaling and Cdo deficient mice exhibit multiple defects in embryonic development, associated with the decreased Shh activity. However its role in heart development is entirely unknown. This study examined the role of Cdo in heart development and cardiomyogenesis using Cdo deficient (*Cdo*^{-/-}) mice. Histological and morphological analyses revealed development of dilated cardiomyopathy (DCM) with a frequent death around 3 weeks of age. Echocardiographic analyses of *Cdo*^{-/-} mice at 2 weeks confirmed the dilated phenotype of the heart with a significant decrease in the left ventricular fractional shortening. Patch clamp experiments revealed an increase in I_{to} and reduction in I_{K1} , without any significant changes in action potential duration (APD) in *Cdo*^{-/-} cardiomyocytes. A major gap junctional protein Connexin 43 (Cx43) appears to be localized at the lateral border of the *Cdo*^{-/-} cardiomyocytes whereas Cx43 remained predominately at the intercalated disk in wildtype cardiomyocytes. Furthermore the phosphorylation status of Cx43 was altered in *Cdo*^{-/-} hearts. Taken together, these data suggest that Cdo signaling may influence the phosphorylation status and distribution of Cx43 thereby regulating intercellular communication and functionality of cardiomyocytes.

1372-Pos Board B323**Loss of PI3K-Gamma Scaffold Function causes Severe Electrical Remodeling in Mice Ventricular Myocytes**Riccardo Rizzetto¹, Alexandra Zahradnikova, Jr.¹, Yueyi Wang¹, Alessandra Ghigo², Rodolphe Fischmeister¹, Emilio Hirsch², Jean-Pierre Benitah¹, Ana M. Gomez¹.¹INSERM UMR-S 769, Laboratory of Signaling and Cardiac Pathophysiology, Université Paris Sud, LabEX LERMIT, Châtenay-Malabry, France, ²Department of Genetics, Biology and Biochemistry, Università degli Studi di Torino, Torino, Italy.

In the heart the kinase/scaffold protein phosphoinositide-3-kinase- γ (PI3K γ) has been associated with maladaptive remodeling. However little is known about the relative contribution of kinase-scaffold roles on the normal electrical activity in cardiac cells.

Here, we compared the action potentials and ionic currents in ventricular myocytes isolated from conventional KO mice or mice expressing a catalytically inactive PI3K ("kinase-dead", KD). Action potentials were evoked at 1Hz (I-clamp). K^+ and L-type Ca^{2+} (I_{CaL}) currents were measured by step depolarizations (V-clamp).

Membrane capacitances (C_m) were not different between genotypes. KO myocytes showed marked prolongation of the action potential duration (APD) at 90% of repolarization (APD₉₀: WT 64.36 \pm 4.1 ms, KO 149.6 \pm 18 ms).